

Basal medium components:	I	II
Advanced DMEM/F-12		
Glutamax	1x	1x
Hepes	10 mM	10 mM
Penicillin Streptomycin	-	1x
Primocin	1x	-

Establishment by digestion								
Digestion mix:	Colon		Stomach		Liver		Pancreas	
	normal	tumorous	normal	tumorous	normal	tumorous	normal	tumorous
Collagenase II	-	-	-	-	-	-	0.625 mg/ml	-
Collagenase XI	-	0.1 mg/ml	-	0.1 mg/ml	-	-	-	-
Collagenase D	-	-	-	-	2.5 mg/ml	-	-	-
Dispase II	-	1 mg/ml	-	1 mg/ml	-	-	2.5 mg/ml	-
DNase I	-	-	-	-	0.1 mg/ml	-	-	-
EDTA	2 mM	-	10 mM	-	-	-	-	-
Diluted in	Chelating buffer*	Basal medium I	Chelating buffer*	Basal medium I	in EBSS		Basal medium I	
Incubation for:	0.5 h on ice	1 h at 37°C	0.25-0.5 h at RT	1 h at 37°C	0.5-1 h at 37°C	1.5 h at 37°C	0.5-1 at 37°C	2-3 h at 37°C
Adapted from reference:	Sato et al. 2011		Bartfeld et al. 2015		Broutier et al. 2016		Hennig et al. 2019	

Cultivation medium (Basic: Basal medium II)				
A83-01	0.5 µM	2 µM	5 µM	0.5 µM
B27	1x	1x	1x	1x
B27 without Vitamin A	-	-	1x	-
Forskolin	-	-	10 µM	-
hFGF-10	-	200 ng/ml	100 ng/mL	100 ng/mL
hHGF	-	-	25 ng/mL	-
[Leu15] Gastrin	10 nM	1 nM	10 nM	10 nM
mEGF	50 ng/mL	50 ng/ml	50 ng/mL	50 ng/mL
N2	-	-	1x	- 1x
N-Acetyl-L-Cystein	1.25 mM	1 mM	1 mM	1 mM
Nicotinamid	10 mM	10 mM	10 mM	10 mM
Noggin	10 %	10 %	-	10 %
Primocin	1x (100 µg/mL)	1x (100 µg/mL)	1x (100 µg/mL)	1x (100 µg/mL)
Prostaglandin E2	10 nM	-	-	- 1 µM
Rspondin	20 %	10 %	10 %	10 %
SB202190	3µM	-	-	-
Wnt3A	50 %	50 %	-	50 %

*Chelating buffer: 5.6 mM Na₂HPO₄, 8.0 mM KH₂PO₄, 96.2 mM NaCl, 1.6 mM KCl, 43.4 mM sucrose, 54.9 mM d-sorbitol, 0.5 mM dl-dithiothreitol in distilled water